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GROWTH OF MOLD ON VEGETABLE DYESTUFFS

By

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ABSTRACT

Molds grow on textiles dyed by vegetable dyestuffs in warm rainy season. A kind of *Penicillium* is isolated from a musty aqueous extract of *Punica granatum L.* The *penicillium* actively grew, and formed spores, on a textiles dyed by vegetable dyestuffs of three kinds of plant tissues, viz., barks of *Myrica rubra* SIEB. & ZUCC., fruits of *Gardenia grandiflora* MAKINO, and fruits of *Punica granatum.* This organism, also, grew in aqueous extract from the plant tissues mentioned above. It grew only a little on the textiles and aqueous extract from wood of *Casesalpinia sappan* L., however. The organism could grew only to whitish colony and not form spores in that extract. The causes of them were investigated. It becomes bow evident that the quantity of thiamine, riboflavin, and other growth factors were insufficient for growth and spore-formation of the organism in aqueous extract of *C. sappan.*

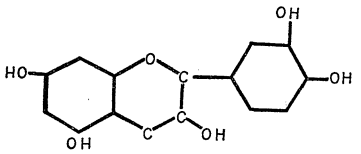
It is frequently found that molds grow in preserved aqueous extract of vegetable dyestuffs. These molds grow even on dyeing textiles at rainy season. There are, however, some differences in growth-activity of the mold owing to the nature of vegetable dyestuffs. The mold fairly grows on dyeing textile by aqueous extract of *Gardenia jasminoides* ELLIS f. *grandiflora* MAKINO, *Punica granatum* L., and *Myrica rubra* SIEB. et ZUCC. On dyeing cloth by *Caesalpinia sappan* L., however, mold grows a little.[1] In this report the authors attend to find some conditions for growth of mold on vegetable dyestuffs.

MATERIAL and METHOD

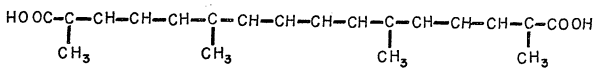
Organism. A mold grown in aqueous extract of *Myrica rubra* SIEB. et ZUCC. was isolated. This organism is a kind of *Penicillium*, and belongs to the species-group of *Monoverticilata.*

Vegetable dyestuffs. Four kinds of plant tissues was used to make culture medium for the organism. They are - 1) cortex of *Myrica rubra* SIEB. et ZUCC. (yellowish colour), 2) fruit of *Punica granatum* L. (yellowish colour), 3) fruit of *Gardenia jasminoides* ELLIS f. *grandiflora* MAKINO (yellowish colour), and 4) wood of *Myrica rubra* SIEB. et ZUCC. (reddish colour).

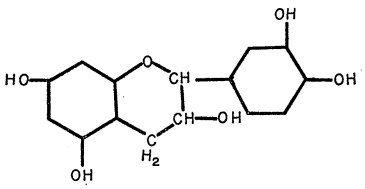
Constitutional formula of colouring matters of four kinds of vegetable dyestuffs[2)]



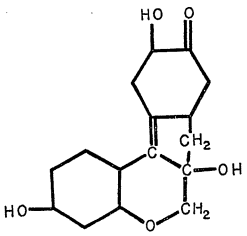
M. rubra



G. grandiflora



P. granatum



C. sappan

Extraction. Each material of dyestuffs was boiled in distiled water for about twenty minutes. The extraction was done in thrice instalments. These parts were mixed up, and then the concentration was made to 3% (w/v).

Culture medium. Aqueous extract of vegetable dyestuffs was used for growth-test of the mold. On liquid medium, 50 ml of the extract was poured into a 300 ml Erlenmeyer flusk. For solid medium, 2% of agar was added to aqueous extract of vegetable dyestuffs. To examine the effect of yeast extract or thiamine and riboflavin on growth of the mold, yeast extract or vitamins was added to aqueous extract of vegetable dyestuffs as shown in the Tables 4, 5 and 6.

Culture. Both shaking and static cultures were used for growth-test of the mold. The shaking culture was carried out by reciprocating shaker. The temperature for culture was 30°C.

Analysis of sugar. The Hanes' method was used for analysis of sugar included in aqueous extract of vegetable dyestuffs. To analyze total sugar, polysaccharide in aqueous extract was hydrolyzed. In 100 ml of aqueous extract 10 ml of 25% hydrochloric acid was added. This mixture was boild for 2.5 hours in water bath. After that the mixture was neutralized with solution of sodium hydroxide. Then the mixture was made up to 100 ml and used for treatment of the analysis.

Determination of thiamine. The potassium ferricyanide and alkali reaction were applicated for the determination of thiamine[3,4,5)].

Reagents for the determination. Isobutyl alcohol; 15% sodium hydroxide; 25% potassium chloride in 0.1 N hydrochloric acid; 1% potassium ferricyanide; potassium ferricyanide alkaline solution; and permutite for the determination of thiamine, the mesh 60-100. It was washed several times by boiling water to remove fluorescence. The permutite was treated with 3% acetic acid, 25% potassium chloride, and boiling water.

Fluoriphotometer.. The Shimazu Fluorophotometer type UM was used. The activate filter was the UV-D_2. The filter cut-420 FL-480 was used as the selection filter[6)].

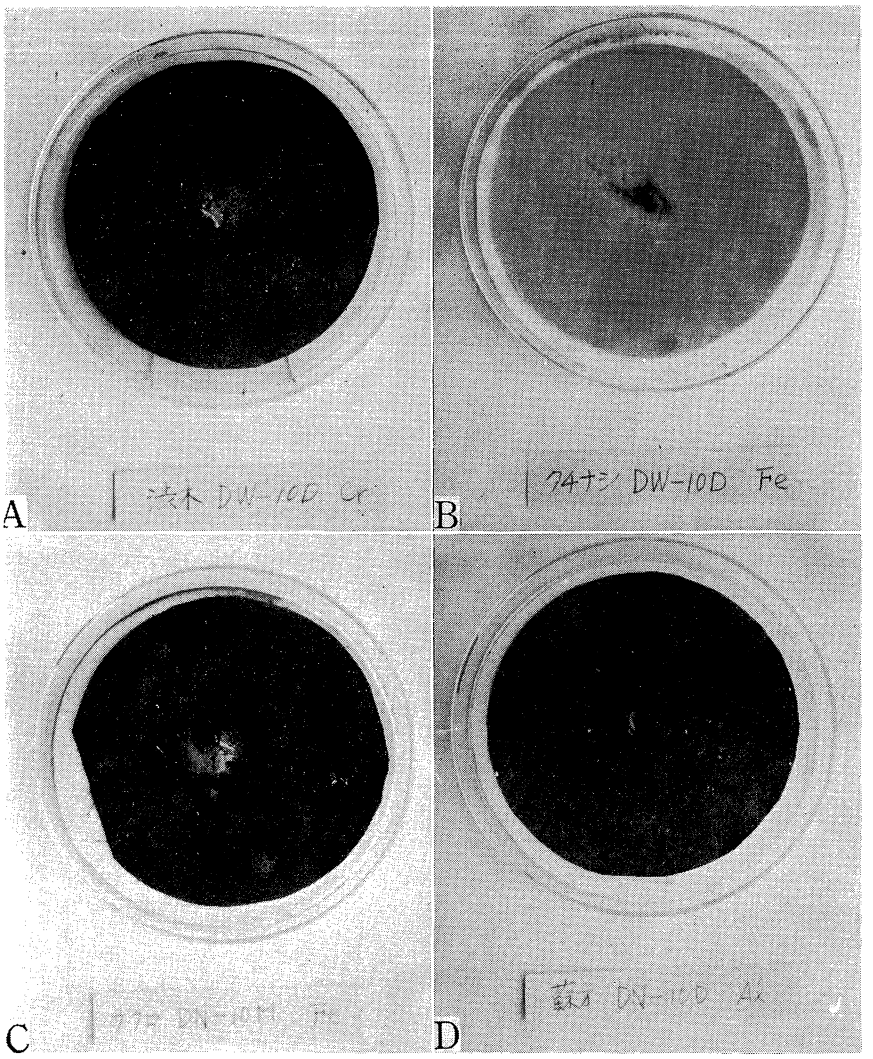


FIG. 1. *Mold grown on the textile dyed by vegetable dyestuffs.*

(A) *The textile dyed by aqueous extract of barks of* Myrica rubra. *The developer is 1 % aqueous solution of potassium bichromata. Whitish colonies are observed in the central surface.*

(B) *The textile dyed by aqueous extract of fruit of* Gardinia grandiflora. *The developer is 1 % aqueous solution of ferric pyroligneous acid. Blackish colonies are observed in central surface.*

(C) *The textile dyed by aqeous extract of fruit of* Punica granatum. *The developer is 1 % of aqueous solution of ferric pyroligneous acid. Whitish colonies are observed in the whole surface.*

(D) *The textile dyed by aqueous solution of wood of* Caesalpinia sappan. *The developer is 1 % of aqueous solution of alum. Any colony are not seen except inoculated mycelium.*

RESULT

Quantity of sugar in aqueous extract of four kinds of plant tissues was analyzed. As the result, considerable quantity of sugar was detected. Quantity of reducing sugar was more than the non-reducing sugar on the three materials, but only in aqueous extract of fruit of *G. grandiflora* the non-reducing sugar had larger quantity (Table 1).

Table 1. *Quantity of sugar in aqueous extract of four kinds of dyestuffs*

	M. rubra	*G. grandiflora*	*P. granatum*	*C. sappan*
	mg/ml	*mg/ml*	*mg/ml*	*mg/ml*
reducing sugar	3.2	1.9	20.6	4.9
non-reducing sugar	0.1	2.2	0.7	0.7

Each aqueous extract was made up from 3% (w/v) of plant tissus.

Various pH values of medium were prepared about the four kinds of extract. On these medium the organism was cultured for 10 days statically. On the extract of *P. granatum* the organism grew most actively among four kinds of medium. And, conspicuous differences of growth activity was not seen in various pH values of medium. On the extract of *M. rubra* and *G. grandiflora* also the organism showed fairly fine growth activity. It was most active at pH value 0.5 or 0 6. Against these phenomena the organism grew just a few on extract of *C. sappan* (Table 2). The aqueous extract of four kinds of plant tissues showed various pH values (Table 3). In general molds can grow agreeably on

Table 2. *Growth of Penicillium in various pH of medium*

organism \ pH		4.5	5.0	5.5	6.0	6.5	7.0
		mg	*mg*	*mg*	*mg*	*mg*	*mg*
M. rubra	dry wt.	21.0	13.6	19.7	34.5	22.8	11.6
	spore formation	++	+	+	+	+	+
G. grandiflora	dry wt.	27.9	28.8	35.5	30.8	25.3	28.1
	spore formation	++	++	++	++	+	+
P. granatum	dry wt.	82.9	67.2	79.4	92.3	89.9	78.2
	spore formation	+++	++	++	++	++	+
C. sappan	dry wt.	7.7	−	7.0	13.6	20.2	8.6
	spore formation	+	−	−	−	−	−

Degree of spore formation was indicated by the symbol of −……white colony, +……whitish green colony, ++……light grayish greencolony, +++……deepgrayish green colony.
The organism was incubated for 10 days on static state.

these pH values. The aqueous extract of wood of *G. grandiflora* showed comparatibly low pH value. But it is considered that the growth of the organism will not be inhibited.

To trace the origin of inhibited growth, yeast extract and glucose was added to basal medium. By addition of yeast extract or glucose the growth of the organism was accelerated. Addition of both yeast extract and glucose bring remarkable stimulation

Table 3. *pH values of aqueous extract of four kinds of dyestuffs*

M. rubra	*G. grandiflora*	*P. granatum*	*C. sappan*
7.39	4.71	5.40	5.08

The extract was made from 3% (w/v) of plant tissues.

Table 4. *The effect of yeast extract and glucose on growth of Penicillium in aqueous extract of dyestuffs*

addition to basal medium	I	II	III	IV
	ml *mg*	*ml* *mg*	*ml* *mg*	*ml* *mg*
yeast extract	0	0.5	1.5	3.0
dry wt.	7.0	19.3	28.5	24.1
glucose	0.5	1.0	3.0	5.0
dry wt.	4.8	10.0	11.3	34.0
yeast extract + glucose	0 / 0.5	0.5 / 1.0	1.5 / 3.0	3.0 / 5.0
dry wt.	5.8	122.5	341.1	101.4

In 6 ml distilled water, 0.5 g of yeast extract was dissolved.
In 10 ml of distilled water, 5.0 g of glucose was dissolved.
The organism was incubated for 14 days on static state.

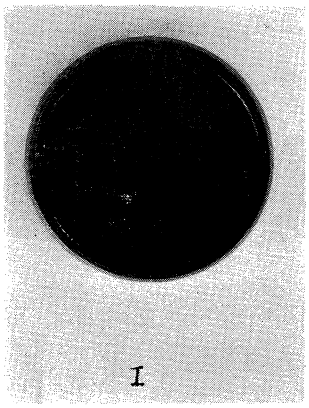


FIG. 2. *Growth of the mold on basal medium made from aqueous extract of* C. sappan. *The colony is scarcely observed.*

Table 5. *The effect of thiamine and riboflavin on growth of Penicillium in aqueous extract of wood of C. sappan*

addition to basal medium	I	II	III	IV	V	VI
	ml mg	*ml mg*	*ml mg*	*ml mg*	*ml mg*	*ml mg*
thiamine	0	0.05	0.1	0.5	1.0	3.0
dry wt.	13.0	18.2	23.3	15.5	—	19.0
rivoflavin		0.05	0.1	0.5	1.0	3.0
dry wt.		13.0	—	17.6	12.7	12.9
thiamine + rivoflavin		0.05 / 0.05	0.1 / 0.1	0.5 / 0.5	1.0 / 1.0	3.0 / 3.0
dry wt.		20.0	22.2	20.7	21.9	23.3

In each flask contain 50 ml of 3% aqueous extract of wood of *C. sappan*, 1.25 g of glucose was supplied.
In 20 ml of aqueous solution of thiamine, 0.5 g of thiamine was contained.
In 20 ml of aqueous solution of riboflavin, 0.5 g of riboflavin was contained.
Each medium was shaked for 7 days by reciprocating shaker on 30°C.

Table 6. *The effect of thiamine and riboflavin on growth of Penicillium in aqueous extract of wood of C. sappan*

addition to basal medium	I	II	III	IV	V	VI
	ml mg	*ml mg*	*ml mg*	*ml mg*	*ml mg*	*ml mg*
thiamine	0	0.05	0.1	0.5	1.0	3.0
dry wt.	7.0	12.7	—	11.4	12.4	11.8
rivoflavin		0.05	0.1	0.5	1.0	3.0
dry wt.		14.2	18.2	14.3	18.5	15.2
thiamine + rivoflavin		0.05 / 0.05	0.1 / 0.1	0.5 / 0.5	1.0 / 1.0	3.0 / 3.0
dry wt.		17.6	18.9	16.7	13.7	—

In each deep Petri dish contain 50 ml of 3 % aqueous extract of wood of *C. sappan*, 1.25 g of glucose was suplied.
In 20 ml of aqueous solution of thiamine, 0.5 g of thiamine was contained.
In 20 ml of aqueous solution of riboflavin, 0.5 g of riboflavin was contained.
The organism was cultured in static state for 12 days on 30°C.

upon the growth (Table 4). To make sure of growth factors contained in yeast extract, thiamine or riboflavin was mixed in basal medium (Tables 5, 6). Both thiamine and riboflavin had effect for growth acceleration in shaking culture. The effect of simultaneous addition of both thiamine and riboflavin was more obvious than the effect of addition

Table 7. *Thiamine contained in aqueous extract of four kinds of plant tissues*

plant tissues	thiamine
	γ/ml
bark of *M. rubra*	22
fruit of *G. grandiflora*	18
fruit of *P. granatum*	8
wood of *C. sappan*	—*

* It could not be detected by use of the fluorophotometer.

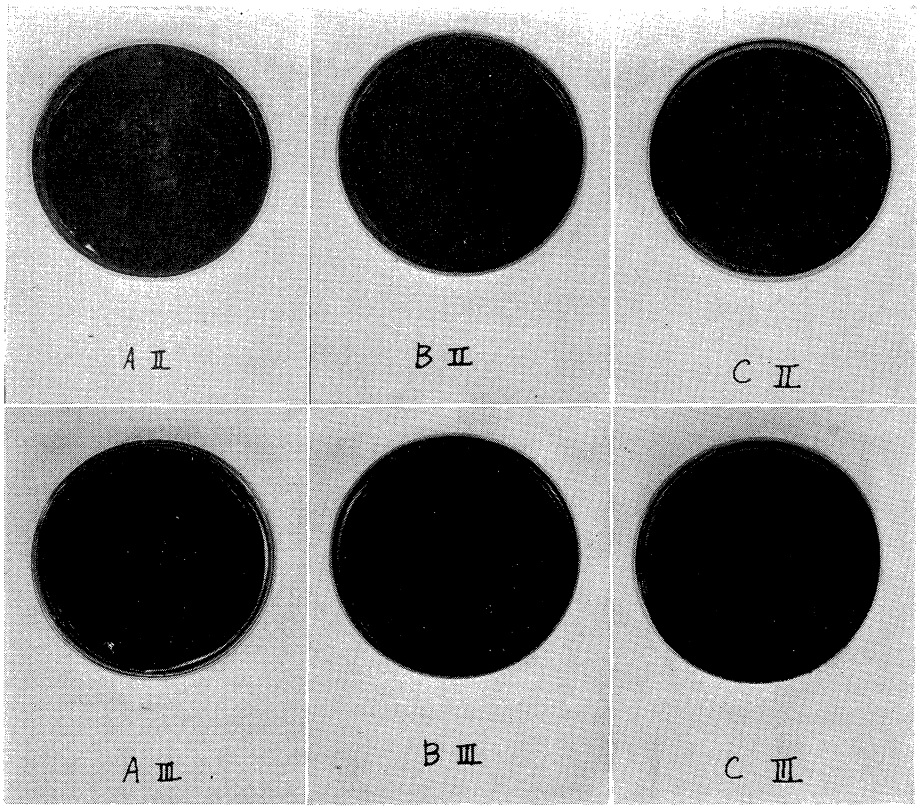


FIG. 3. *Growth of the mold on medium enriched by thiamine or riboflavin.* (A) *enriched by thiamine*; (B) *enriched by riboflavin*; (C) *enriched by both thiamine and riboflavin*; (II) *enriched by 0.05 ml of 10 % (w/v) aqueous solution of growth factor*; (III) *enriched by 0.1 ml of 10 % (w/v) aqueous solution of growth factor.*

of individual vitamin (Table 5). On the contrary in static culture only a little effect of addition was given (Table 6).

Thiamine contained in 3% aqueous extract of *C. sappan* was measured. A fairly sufficient thiamine was detected in aqueous extract of *M. rubra*, *G. grandiflora* and *P. granatum*. But in extract of *C. sappan* thiamine was not detected (Table 7).

Discussion

Various molds grow and form spores on aqueous extract of plant tissues available as vegetable dyestuffs. Aqueous extract of bark of *M. rubra*, fruit of *G. grandiflora*, fruit of *P. granatum* and wood of *C. sappan* are worthy as fairy good nutrient souce for *Penicillium*. But the colouring matters originated in these plant tissues have comparatively stable nature for biological action. So it is considerable that there may be some more nutritious substances in these aqueous extract.

The *Penicillium* showed good growth in aqueous extract of *M. rnbra*, *G. grandiflora* and *P. granatum*. But the organism grew only a little in spite of including fairly reduced sugar. It is considerable that the inhibition of growth is due to a lack of growth factors.

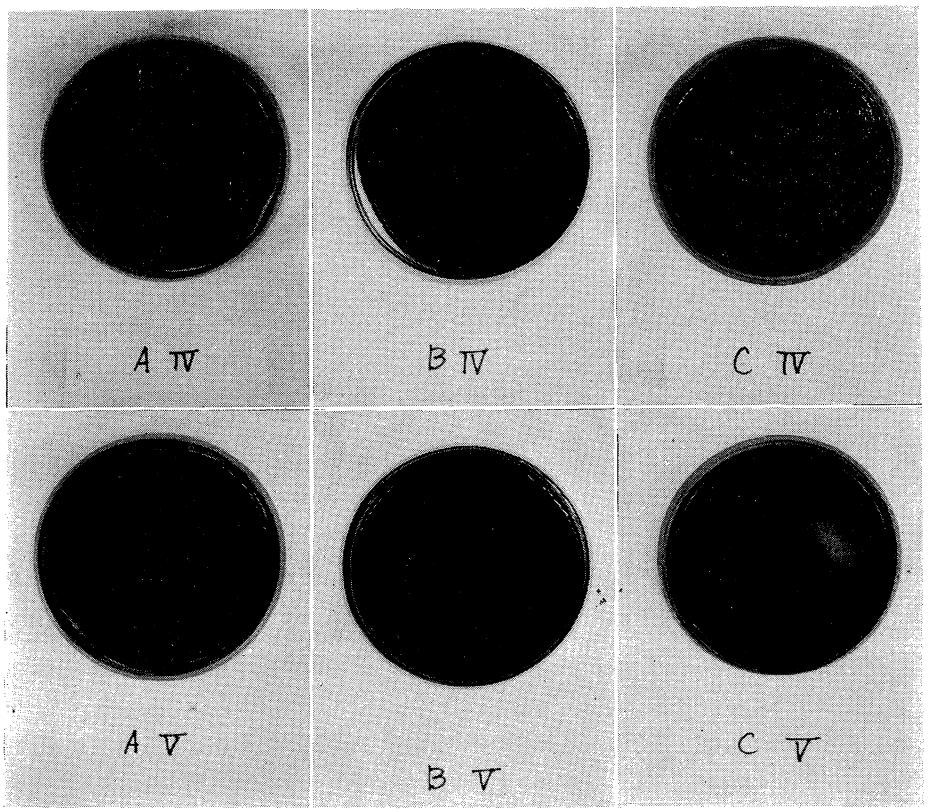


FIG. 4. *Growth of the mold on medium enriched by thiamine or riboflavin.* (IV) *enriched by 0.5 ml of growth factor solution* ; (V) *enriched by 1.0 ml of growth factor solution.*

This opinion was proved by the results shown on the Table 4, 5, 6 and 7. Perhaps the organism will require many kinds of growth factors to grow in extract of the plant tissues in spite of having ability on some growth factors synthesis. Only the effects of thiamine and rboflavin on growth of the organism were tested and thiamine was measured on this experiment. A reinvestigation of effects and measurement of the other vitamin is necessary.

The organism showed a characteristic at the growth on aqueous extract of *C. sappan*. It grew to whitish colony. In this case, spores are scarcely formed. If yeast extract or both thiamine and riboflavin is given to *C. sappan* extract, spores are formed plentifully and the colony grew to grayish green. For this organism some growth factors to form spores must be lack in *C. sappan* extract.

Acknowledgments

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